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<p>(21) International Application Number: PCT/US94/02964 (22) International Filing Date: 23 March 1994 (23.03.94) (30) Priority Data: 08/036,378 24 March 1993 (24.03.93) US (71) Applicant: THE DU PONT MERCK PHARMACEUTICAL COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventor: KETTNER, Charles, Adrian; 2411 Chatham Drive, Wilmington, DE 19803-2706 (US). (74) Agents: REINERT, Norbert, F. et al.; The Du Pont Merck Pharmaceutical Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).</p>		<p>(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: REMOVAL OF BORONIC ACID PROTECTING GROUPS BY TRANSESTERIFICATION</p> <p>(57) Abstract</p> <p>A method for the removal of ester protecting groups from α-amino boronic acid is disclosed for the preparation of compounds of the formula: $R^1-X_2-NHCH(R^2)-B(OH)_2$.</p> <p style="text-align: center;">BEST AVAILABLE COPY</p>		

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-1-

TitleRemoval of Boronic Acid Protecting Groups by
Transesterification

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Field of the Invention

The present invention relates to a process for the removal of ester protecting groups from α -amino boronic acids and corresponding peptide analogs by
10 transesterification with hydrophobic boronic acids.

Background of the Invention

Simple boronic acids are inhibitors of serine proteases. For example, Koehler et al. *Biochemistry* 10:
15 2477 (1971) reports that 2-phenylethane boronic acid inhibits chymotrypsin at millimolar levels. The synthesis of boronic acid analogs of N-acyl- α -amino acids has yielded more effective inhibitors. Ac-boroPhe-OH, R-1-acetamido-2-phenylethane boronic acid,
20 inhibits chymotrypsin with a K_i of 4 μ M Matteson et al. *J. Am. Chem. Soc.* 103: 5241 (1981). More recently, Shenvi, US 4,537,773 (1985) disclosed that boronic acid analogs of α -amino acids, containing a free amino group, were effective inhibitors of aminopeptidases. Shenvi,
25 US 4,499,082 (1985) discloses that peptides containing an α -amino boronic acid with a neutral side chain were more effective inhibitors of serine proteases exceeding inhibitors disclosed earlier by as much as 3 orders of magnitude in potency. The chemistry of α -aminoboronic
30 acids was further expanded to the synthesis of peptide analogs containing boronic acid with positive charged side chains, borolysine, boroArginine, boroOrnithine, and isothiuronium analogs. This is disclosed in Kettner, et al. EPA 0,293,881, published December 7,
35 1988.

Much progress has been made in the synthesis of boronic acid and corresponding peptides with the

-2-

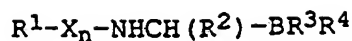
boronic acid protected as an ester. However, a convenient method of removal of the ester protecting group is lacking. Matteson (1981) *infra*, reports the destructive removal of pinanediol group by treatment with anhydrous BCl_3 . Kettner and Shenvi *J. Biol. Chem.* 259: 15106 (1984) describe the removal of the pinacol protecting group by converting the boronic pinacol esters to the thermodynamically more stable, diethanolamine ester by transesterification and then hydrolysis by treatment with aqueous acid or with a cation exchange resin. This method is not applicable for removal of pinanediol ester due to its greater stability. Matteson *Chem. Rev.* 89: 1535 (1989) describes the removal of the pinanediol group *in situ* by incubations in borate buffer. It should be noted that the pinanediol ester is preferred in synthesis due to its ability to direct stereochemistry at the α -carbon of boronic acid and its stability to chemical manipulations. The pinanediol protecting group was used almost exclusively in the preparation of boroArginine peptides, shown in EPA 0,293,881. In one example, partial hydrolysis of the pinanediol ester was obtained by binding Ac-(D)Phe-Pro-boroArg- $\text{C}_{10}\text{H}_{16}$ to a cation exchange resin and washing extensively with aqueous acetic acid followed by elution with HCl . This reaction is slow, it requires recovery of product by evaporation of large volumes of water and separation of the free boronic acid from the ester. Removal of the pinanediol by treatment with BCl_3 as the final step in synthesis was considered to be the only practical method.

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Summary of the Invention

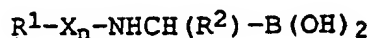
The present invention provides a method for converting compounds of formula I

-3-



(I)

5 to compounds of formula II,



(II)

wherein for both formula I and formula II

10 R^1 is

- a) hydrogen,
- b) an N-terminal protecting group,
- c) $-SO_2(CH_2)_m$ -aryl, wherein aryl is phenyl, naphthyl or biphenyl substituted with one, two or three
- 15 substituents selected from the group consisting of halo (F, Cl, Br, I), $-CN$, C1-C10-alkyl, C3-C8-cycloalkyl, C2-C10-alkenyl, C2-C10-alkynyl, $-OR^7$, $-NO_2$, $-CF_3$, $-S(O)_rR^8$, $-NR^6R^7$, $-COR^7$, $-CO_2R^7$, $-CONR^6R^7$;

X is a peptide of 1-20 amino acids;

20 R^2 is

- a) C1-C10-alkyl,
- b) C2-C10-alkyl-Y,
- c) $-(CH_2)_n$ -aryl, wherein aryl is as defined above;

Y is

- 25 a) $-NHC(NH)NH_2$,
- b) $-NH_2$,
- c) $-SC(NH)NH_2$,
- d) $-OR^9$,
- e) $-SR^9$;

30 R^3 and R^4 are

- a) C1-C8-alkoxy, or
- b) when taken together R^3 and R^4 form a cyclic boronic ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, a heteroatom which
- 35 can be N, S, or O;

R^5 and R^6 are independently

- a) H,

-4-

- b) C1-C8-alkyl,
- c) C1-C8-alkoxy,
- d) C3-C8-cycloalkyl,
- e) $-\text{CO}_2\text{R}^7$,
- 5 f) $-(\text{CH}_2)_m\text{-phenyl}$;

R^7 is

- a) H,
- b) phenyl,
- c) benzyl,
- 10 d) C1-C8-alkyl;

R^8 is

- a) phenyl,
- b) C1-C4-alkyl,
- c) C1-C4-alkoxy,
- 15 d) $-\text{CF}_3$;

R^9 is

- a) H,
- b) C1-C2-alkyl,
- c) phenyl or phenyl optionally substituted with a
- 20 substituent selected from the group consisting of halo (F, Cl, Br, I), $-\text{CN}$, C1-C10-alkyl, C3-C8-cycloalkyl, C2-C10-alkenyl, $-\text{C}_2\text{-C}_{10}\text{-alkynyl}$, $-\text{OR}^7$, $-\text{NO}_2$, $-\text{CF}_3$, $-\text{S}(\text{O})_r\text{R}^8$, $-\text{NR}^6\text{R}^7$, $-\text{COR}^7$, $-\text{CO}_2\text{R}^7$, $-\text{CONR}^6\text{R}^7$ wherein R^5 , R^6 , and R^8 are as defined above;
- 25 n is 0 or 1;
- m is 0 to 2;
- r is 0 to 2;

which comprises reacting a compound represented by

30 formula I in a mixture of water and a water-immiscible organic solvent containing an organic boronic acid acceptor present in an amount equal to at least 1 equivalent of the compound of formula I, stirring the mixture at a temperature in a range of from about 5 to

35 about 35°C , for a time of approximately 1 hour, allowing the mixture to then separate into two distinct phases,

-5-

separating the phases and then recovering the desired compound of formula II from the separated aqueous phase.

5 Detailed Description of the Invention

As used throughout the specifications, the following abbreviations for amino acid residues or amino acids apply:

	Ala	=	L-alanine
10	Arg	=	L-arginine
	Asn	=	L-asparagine
	Asp	=	L-aspartic acid
	Cys	=	L-cysteine
	Gln	=	L-glutamine
15	Glu	=	L-glutamic acid
	Gly	=	glycine
	His	=	L-histidine
	Ile	=	L-isoleucine
	Leu	=	L-leucine
20	Lys	=	L-lysine
	Met	=	L-methionine
	Phe	=	L-phenylalanine
	Pro	=	L-proline
	Ser	=	L-serine
25	Thr	=	L-threonine
	Trp	=	L-tryptophan
	Tyr	=	L-tyrosine
	Val	=	L-valine

The "D" prefix for the foregoing abbreviations indicates
30 the amino acid is in the D-configuration. "D,L"
indicates the amino is present in mixture of the D- and
the L-configurations.

Other abbreviations used throughout the description
35 below

are:

Me = methyl

-6-

	Et	= ethyl
	Boc	= t-butoxycarbonyl
	Z	= benzyloxycarbonyl
	2Clz	= 2-chlorobenzyloxycarbonyl
5	4Clz	= 4-chlorobenzyloxycarbonyl
	p-NO ₂ -Z	= p-NO ₂ benzyloxycarbonyl
	AC	= acetyl
	Adc	= adamantyloxycarbonyl
	DIPA	= diisopropylamine
10	DIPEA	= diisopropylethylamine
	DCHA	= dicyclohexylamine
	DBU	= 1,8-diazabicyclo[5.4.0]undec-7-ene
	DABCO	= 1,4-diazabicyclo[2.2.2]octane
	NMM	= N-methylmorpholine
15	DMAP	= 4-dimethylaminopyridine
	FSA	= formamidinesulfinic acid
	FAB/MS	= fast atom bombardment mass
	spectrometry	MS (NH ₃ -Cl) = chemical
	ionization mass spectrometry	
20	NMR	= nuclear magnetic resonance
	spectrometry	

The following reagents were obtained from commercial sources: 1-hydroxybenzotriazole·H₂O, adamantylfluoroformate, di-t-butylidicarbonate, benzyloxycarbonyl chloride, 2-chlorobenzyloxycarbonyl chloride, N-hydroxysuccinimide, formamidinesulfinic acid, 32% peracetic acid.

Boc-Pro-boroOrn-C₁₀H₁₆, Ac-(D)Phe-Pro-boroOrn-C₁₀H₁₆, BocPhe-boroOrn-C₁₀H₁₆ benzenesulfonic acid were prepared by the procedure described in EP0293881A2, p12-13.

The prefix "boro" indicates amino acid residues where the carboxy group is replaced by a boronic acid (formula II, R³ and R⁴ = -OH).

The pinanediol boronic acid ester and the pinacol boronic acid ester are abbreviated "-C₁₀H₁₆" and "C₆H₁₂",

-7-

respectively. Other illustrations of diols useful for deriving a boronic acid esters are 1,2-ethanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, 1,2-dicyclohexylethanediol.

5 Note that throughout the text when an alkyl substituent is mentioned, the normal alkyl structure is meant (e.g. butyl is n-butyl) unless otherwise specified. However, in the definition of radicals above (e.g. R^2), both branched and straight chains are
10 included in the scope of alkyl.

 It is understood that many of the compounds of the present invention contain one or more chiral centers and that these stereoisomers may possess distinct physical and
15 biological properties. The present invention comprises all of the stereoisomers or mixtures thereof. If the pure enantiomers or diastereomers are desired, they may be prepared using starting
15 materials with the appropriate stereochemistry, or
20 may be separated from mixtures of undesired stereoisomers by standard techniques, including chiral chromatography and recrystallization of diastereomeric salts.

 "N-terminal protecting group" as used herein,
25 refers to various art recognized amino-terminal protecting groups employed in peptide synthesis. Examples of suitable groups include formyl, acetyl, benzoyl, trifluoroacetyl, and methoxysuccinyl; aromatic urethane protecting
30 groups, such as, benzyloxycarbonyl; and aliphatic urethane protecting groups, such as t-benzyloxycarbonyl or adamantyloxycarbonyl. Gross and Meinhoffer, eds., The Peptides, Vol. 3; 3-88 (1981), Academic
35 Press, New York 1981, disclose numerous suitable amine protecting groups and is incorporated herein by reference for

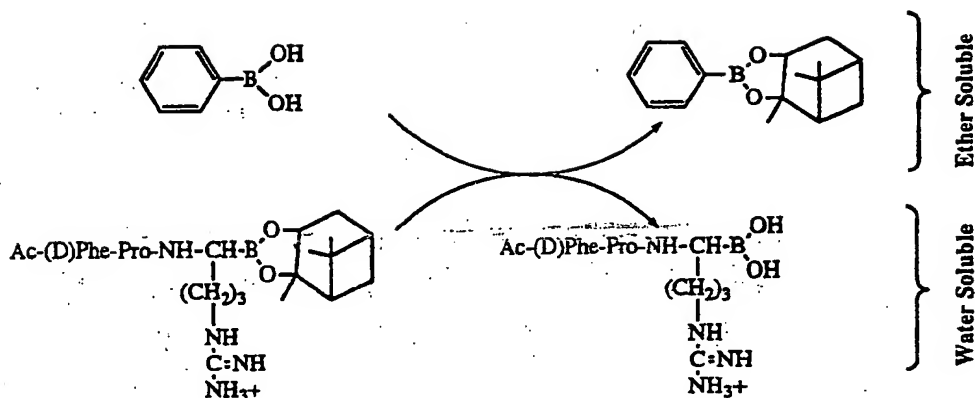
that purpose.

"Peptide of 1-20 amino acids" as used herein, refers to a peptide chain of one to twenty natural or unnatural amino acids of either D- or L-configuration. Roberts and Vellaccio, The Peptides, Vol. 5; 341-449, Academic Press, New York 1983, disclose numerous suitable natural and unnatural amino acids and is incorporated herein by reference for that purpose. This term is also intended to include sidechain protected amino acid residues that are commonly employed in peptide synthesis such as those disclosed in the Peptides, Vol 3, 3-88 (1981). This reference is incorporated herein by reference for that purpose.

It should be noted that to yield a compound of formula II where X is a peptide, optionally, the N-terminal or sidechain protecting groups can be removed by using procedures well known to those skilled in the art. For example, where the N-terminal or side chain protecting group is BOC, the BOC group can be removed by treatment with Anhydrous HCL. Where the N-terminal or side chain protecting group is Z, the Z group can be removed by means of catalytic hydrogenation.

The present invention relates to the synthesis of free boronic acids (compounds of formula II) from ester precursors by transesterification reactions with aliphatic and aromatic boronic acids under heterogeneous reaction conditions.

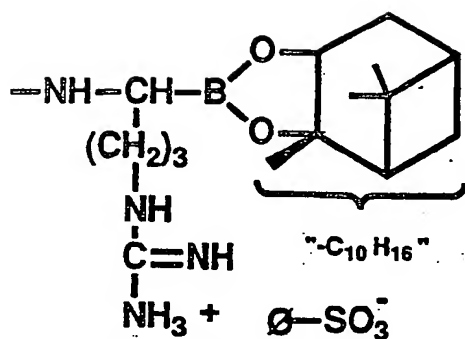
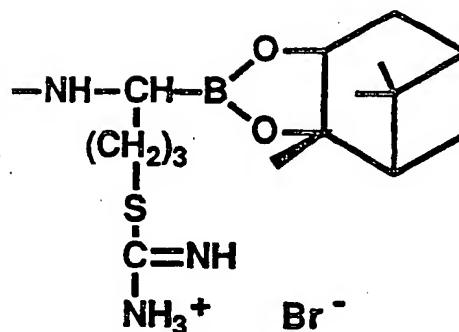
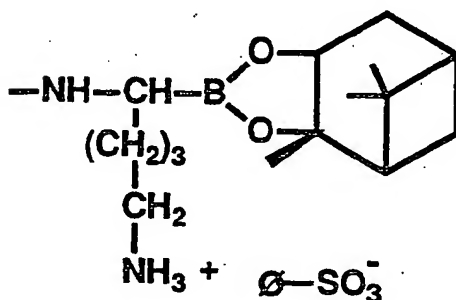
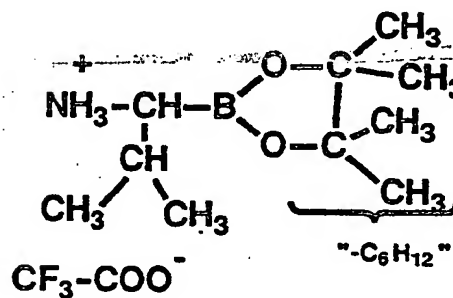
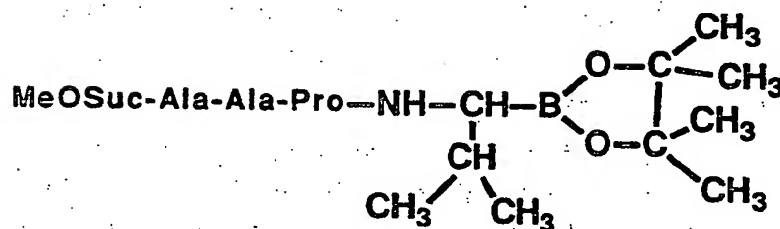
5 Scheme 1



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This novel method is readily applicable to compounds where the R² side chain is positively charged as shown in Scheme 1 where R² is the 3-guanidino-propyl moiety. In this example, the protected boronic acid ester, Ac-(D)Phe-Pro-boroArg-C₁₀H₁₆, is suspended in a mixture consisting of water, an equal volume of diethyl ether, and 5 equivalents of phenyl boronic acid. The flask is stoppered and allowed to stir rapidly with a magnetic stirrer at room temperature. Two clear phases are observed after 15-30 min. Stirring is continued for 3 hr. The reaction mixture is transferred to a separatory funnel where the phases are separated. The aqueous phase is then washed with two portions of ether. Water is removed by evaporation at 35-43°C at a reduced pressure. Products are usually obtained as white foams after drying in vacuo with KOH and P₂O₅ and are readily converted to amorphous white solids by triturating with ether.

-10-

boroArg-C₁₀H₁₆Isothiuronium Salt
Analog of boroArg-C₁₀H₁₆boroLys-C₁₀H₁₆H-boroVal-C₆H₁₂MeOSuc-Ala-Ala-Pro-(D,L)boroVal-C₆H₁₂

The above process depends on the final product being more soluble in the aqueous phase than the organic phase. This criteria is readily met for compounds such as the boroArginine, boroLysine, and boroOrnithine peptides as well as analogs where the isothiuronium group replaces the guanidino group. It is applicable to compounds in US 4,537,773 and US 4,499,082 which describe α-aminoboronic acids with neutral side chains

-11-

and peptides containing α -aminoboronic acids with neutral side chains, respectively. For removal of the ester protecting group from α -aminoboronic such as H-boroVal-C₆H₁₂, this method should be generally applicable since these compounds are readily soluble in water due to the presence of the free α -amino group. It should be applicable to a large number of less hydrophobic peptide boronic acids which are readily soluble in water. For example, the pinacol protecting group of MeOSuc-Ala-Ala-Pro-boroVal-OH is readily removed by the method of the present invention. However, it will be desirable to run trial reactions on a small scale to determine the solubility of the free boronic acid product and the feasibility of this method. For more hydrophobic compounds in this series, it maybe necessary to design a synthetic protocol were the transesterification step is applied to intermediates containing charged residues.

The use of a biphasic system with the organic phase consisting of diethyl ether and phenyl boronic appears to be ideal for the preparation of most free boronic acids. This method will be applicable to the removal of other boronic acid protecting groups represented by R³ and R⁴ in formula (I). Specific examples, in addition to the pinanediol and pinacol groups, are where R³ and R⁴ taken together form a moiety derived from 1,2-ethanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, or 1,2-dicyclohexylethanediol. The protecting groups can also be where R³ and R⁴ are derived from alcohols such as isopropanol, methanol, ethanol or n-propanol. Of course, R³ and R⁴ can each be derived from the same alcohol or from different alcohols, if desired.

Organic solvents other than diethyl ether can be used in the method of the invention. It is only necessary that the organic solvent be water immiscible. Suitable choices of other organic solvents are

-12-

carbonteterachloride, chloroform, methylenechloride, ethyl acetate, benzene, tolulene or hexane.

Boronic acid acceptors for the ester protecting group other than phenyl boronic acid also can be used in the method of the invention. It is only necessary that the acceptor boronic acid, both in its free form and in its esterified form, have greater solubility in the organic phase than in the aqueous phase. Suitable choices of other acceptor boronic acids are butyl boronic acid, pentyl boronic acid, hexyl boronic acid or cyclohexyl boronic acid.

For the method of invention, the ratio of water to organic solvent in the mixture in which the ester precursor of formula (I) is suspended can vary widely. It is important that sufficient volumes of water and organic solvent be present to completely dissolve the products of the reaction (acceptor boronic acid plus ester for the organic phase and free boronic acid for the aqueous phase).

For the method of the invention, the amount of acceptor boronic acid in the reaction mixture should be an amount equal to at least a molar equivalent of the ester precursor of formula (I) present in said mixture. Generally, it is preferable to have the acceptor boronic acid present in an amount in excess of an equimolar amount, the most preferred amount-being a range of from 3 to 5 equivalents.

The time of stirring the reaction mixture can vary over wide limits depending on the ester precursor and the acceptor boronic acid involved. Usually, the minimum time for stirring is 1 hour, but can vary from 0.2 to 48 hours.

In the method of the invention, the desired product compound of formula (II) is recovered from the aqueous phase after its separation from the two phase system formed from stirring the reaction mixture. This

-13-

is best accomplished by the removal of water from the aqueous phase by means well understood by those skilled in the art, such as with a rotary evaporator.

NMR, proton nuclear magnetic resonance, chemical shifts are reported in δ units, parts per million downfield from the internal tetramethylsilane standard. Elemental analyses were conducted by Galbraith Laboratories Inc., Knoxville, TN and Microanalysis Inc., Wilmington, DE. FAB/MS samples of free boronic acids did not give consistent results making it difficult to monitor the removal of ester protecting groups difficult by this means. However, the presence of the pinanediol and the pinacol groups are readily observed in NMR spectra. For the pinanediol ester, a methyl group is observed at δ 0.9 and the methyl groups of the pinacol groups are observed as singlet at δ 1.1. Following the removal of pinanediol protecting group, FAB/MS were run by treating the sample with ~2 equivalents of pinacol in methanol for 5 min and evaporating the solvent. Similarly, FAB/MS samples of free boronic acid, obtained by removal of the pinacol, were prepared by treating with pinanediol.

Example 1

Preparation of Ac-(D)Phe-Pro-boroArg-OH•benzene, sulfonic acid.

The synthesis of Ac-(D)Phe-Pro-boroArg-C₁₀H₁₆•benzene sulfonic acid has been described previously, Kettner et al. *J. Biol Chem* 265: 18289 (1990).

Ac-(D)Phe-Pro-boroArg-C₁₀H₁₆•benzene sulfonic acid (0.20 g, 0.27 mmols) and phenyl boronic acid (0.16 g, 1.3 mmols) were suspended in a mixture consisting of 5 ml of water and 5 ml of ether. The mixture was stirred overnight at room temperature. The two phases were separated, the organic phase was washed with water, and the aqueous phase was washed with ether. The combined

-14-

aqueous phases was evaporated to yield 0.14 g of product. NMR was consistent with the desired structure and the product obtained in Example 2.

5

Example 2

Preparation of Ac-(D)Phe-Pro-boroArg-OH·HCl, Ac-(D)Phe-Pro-boroArg-C₁₀H₁₆·benzene sulfonic acid (6.4 g, 8.5 mmols) and phenyl boronic acid (5.2 g, 42 mmols) were suspended in 150 ml of water and 150 ml of ether. The mixture was stirred overnight. The phases were separated and the ether phase was washed with two 100 ml portions of water. The combined aqueous phases were washed with ether. The aqueous phase was concentrated to ~50 ml by evaporation and then it was passed through a column containing 15 ml of BioRad™ AG1-X8 (Cl⁻ form). The aqueous phase was further concentrated to ~2 ml and it was chromatographed on a 2.5 x 100 cm column containing BioRad™ P2 resin and equilibrated with 1.0 mM HCl. Fractions containing the desired product were pooled, evaporated, dried in vacuo, and triturated with ether to yield 3.4 g.

Anal. Calcd. for C₂₁H₃₄N₆O₅BCl: C= 50.77%, H=6.91%, N=16.92%, and B=2.18%. Found: C=50.91%, H=6.97%, N=16.91%, B=2.29%.

25

Example 3

Preparation of Ac-Phe-Pro-boroArg-OH·HCl
The starting material for this reaction, Ac-Phe-Pro-boroArg-C₁₀H₁₆·HCl, was prepared by coupling Ac-Phe-OH to H-Pro-boroArg-C₁₀H₁₆. The intermediate Boc-Pro-boroOrn-C₁₀H₁₆ was prepared by the procedure described in EPA 0 293 881 and it was guanidated using aminoiminomethane sulfonic acid [Mosher et al. *Tetrahedral Lett.* **29**: 3183 (1988)]. Boc-Pro-boroOrn-C₁₀H₁₆·benzene sulfonic acid (4.8 g, 10.4 mmols) was

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-15-

dissolved in 50 ml of absolute ethanol; 4-dimethylaminopyridine (2.5 g, 20.7 mmoles) and aminoiminomethane sulfonic acid (2.6 g, 20.7 mmoles) were added. The mixture was refluxed at 80°C for 3 hrs.

5 It was cooled and solids were removed by filtration. Solvent was evaporated, the residue was dissolved in chloroform, and it was washed with 0.2 N HCl prepared in saturated aqueous NaCl and with saturated aqueous NaCl. After drying over anhydrous sodium sulfate, solvent was
10 evaporated to yield 5.4 g of a foam. This material was dissolved in methanol and it was chromatographed on a 2.5 x 100 cm column of Sephadex™ LH-20 using methanol as a solvent. Product, 4.4 g, was obtained. FAB/MS calcd. for M (C₂₅H₄₄N₅O₅B) + H: 506.56. Found: 506.49.

15 H-Pro-boroArg-C₁₀H₁₆•2HCl was prepared by dissolving Boc-Pro-boroArg-C₁₀H₁₆•HCl (1.3 g, 2.4 mmoles) in 10 ml of dioxane and adding 10 ml of 3.3 N HCl: dioxane. After stirring for 2 hrs, solvent was evaporated and the residue was triturated with ether to
20 yield 1.2 g of product. FAB/MS calcd. for M (C₂₀H₃₆N₅O₃B) + H: 406.43. Found: 406.38.

Ac-Phe-OH (87 mg, 0.42 mmoles) was coupled to H-Pro-boroArg-C₁₀H₁₆•2HCl (200 mg, 0.42 mmoles) using the carbodiimide procedure. The starting materials were
25 dissolved in 20 ml of methylene chloride, N-methylmorpholine (92 µl, 0.84 mmoles), 1-hydroxybenzotriazole•H₂O (130 mg, 0.84 mmoles), and dicyclohexylcarbodiimide (86 mg, 0.42 mmoles) were added. After stirring overnight at room temperature,
30 the reaction mixture was filtered, the filtrate evaporated, and the residue was chromatographed 2.5 x 50 cm column of LH-20 using methanol as a solvent. The desired product was obtained in a yield of 240 mg. FAB/MS calcd. for M (C₃₁H₄₇N₆O₅B) + H: 595.66. Found:
35 595.41.

Ac-Phe-Pro-boroArg-C₁₀H₁₆•HCl (0.13 g, 0.21 mmoles) and phenyl boronic acid (0.13 g, 1.0 mmoles)

-16-

were dissolved in a mixture of 5 ml of water and 5 ml of ether. The mixture was stirred 3 hrs at room temperature. The reaction phases were separated and the aqueous phase was extensively washed with ether. Water was evaporated and the residue dried to yield 0.11 g. The product was triturated with ether to yield a white solid. FAB/MS calcd. for the pinacol ester, M ($C_{27}H_{43}N_6O_5B$) + H: 543.58. Found: 543.48.

10

Example 4

Preparation of Ac-Pro-boroArg-OH·HCl

Ac-Pro-boroArg- $C_{10}H_{16}$ ·HCl was prepared by dissolving H-Pro-boroArg- $C_{10}H_{16}$ ·2HCl (200 mg, 0.41 mmoles) in 1 ml of dioxane: water (1:1) and adding acetic anhydride (59 μ l, 0.63 mmoles) and sodium bicarbonate (110 mg, 1.2 mmoles). The reaction was allowed to stir 30 min at room temperature, it was acidified with HCl, diluted with methanol, and evaporated. It was redissolved in methanol and chromatographed on 2.5 x 50 cm column of LH-20. Fractions containing the desired product were pooled, evaporated, and triturated with ether to yield 140 mg. FAB/MS calcd. for M ($C_{22}H_{38}N_5O_4B$) + H: 447.97. Found: 448.43.

25

The conditions in Example 3 were used to prepare the free boronic acid of Ac-Pro-boroArg- $C_{10}H_{16}$ ·HCl (0.12 g, 0.24 mmoles). After triturating the product with ether, 0.080 g of Ac-Pro-boroArg-OH·HCl were obtained. FAB/MS calcd. for the pinacol ester, M ($C_{18}H_{34}N_5O_4B$) + H: 396.39. Found: 396.3.

30

Example 5

Preparation of Ac-Gly-boroArg-OH·benzene sulfonic acid

Boc-Gly-boroArg- $C_{10}H_{16}$ (10.2 g) was prepared from Boc-Gly-boroOrn- $C_{10}H_{16}$ ·benzene sulfonic acid (12.5 g, 21.5 mmoles) by the procedure described in EPA 0 293

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-17-

881. FAB/MS calcd. for M (C₂₂H₄₀N₅O₅B) + H: 466.32.

Found: 466.59.

H-Gly-boroArg-C₁₀H₁₆•HCl, benzene sulfonic acid was prepared by deblocking Boc-Gly-boroArg-C₁₀H₁₆ with

5 HCl: dioxane.

Ac-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid was prepared by the procedure described for Ac-Pro-boroArg-C₁₀H₁₆ in Example 4. FAB/MS calcd. for M (C₁₉H₃₄N₅O₄B) + H: 407.90. Found: 408.36.

10 The condition described for Example 3 were used to prepare the free boronic acid. Ac-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid (0.064 g, 0.11 mmoles) yielded 33 mg of Ac-Gly-boroArg-OH•benzene sulfonic acid. FAB/MS calcd. for the pinacol ester, M
15 (C₁₅H₃₀N₅O₄B) + H: 356.32. Found: 356.3.

Example 6

Preparation of Ac-(D)Phe-Gly-boroArg-OH•benzene sulfonic acid

20 Ac-(D)Phe-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid was prepared by coupling Ac-(D)Phe-OH to H-Gly-boroArg-C₁₀H₁₆ using a modification of the carbodiimide procedure described in Example 3. For this coupling, 2 ml of dimethylformamide was used with 20 ml of methylene
25 chloride as a solvent. FAB/MS calcd. for M (C₂₈H₄₃N₆O₅B) + H: 555.59. Found: 555.38.

The procedure described in Example 3 was used to prepare the free boronic acid. Ac-(D)Phe-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid (0.10 g, 0.14 mmoles)
30 yielded 72 mg of Ac-(D)Phe-Gly-boroArg-OH•benzene sulfonic acid. FAB/MS calcd. for the pinacol ester M (C₂₄H₃₉N₆O₅B) + H: 503.51. Found: 503.32.

Example 7

35 Preparation of Boc-(D)Phe-Gly-boroArg-OH•HCl
Boc-(D)Phe-Gly-boroArg-C₁₀H₁₆ was prepared by coupling Boc-(D)Phe-OH to the dipeptide analog using the

-18-

mixed anhydride procedure. The mixed anhydride of Boc-(D)Phe-OH (95 mg, 0.36 mmoles) was prepared by dissolving the acid in 3 ml of anhydrous tetrahydrofuran and adding N-methylmorpholine (40 μ l, 0.36 mmoles), and isobutyl chloroformate (46 μ l, 0.36 mmoles) at -20°C. After 5 min, triethylamine (50 μ l, 0.36 mmoles) and 10 ml of cold tetrahydrofuran were added and the mixture was immediately added to a 0°C solution of H-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid, HCl (200 mg, 0.36 mmoles) in 6 ml of chloroform. After allowing the reaction to warm to room temperature and to stir several hrs, it was filtered and solvent was evaporated. The residue was chromatographed on a 2.5 x 50 cm column of LH-20 in methanol to yield 210 mg of the desired product. FAB/MS calcd. for M (C₃₁H₄₉N₆O₆B) + H: 613.39. Found: 613.65.

The procedure described in Example 3 was used to convert Boc-(D)Phe-Gly-boroArg-C₁₀H₁₆•HCl (0.050 g, 0.077 mmoles) to 36 mg of Boc-(D)Phe-Gly-boroArg-OH•HCl. FAB/MS calcd. for the pinacol ester, M (C₂₇H₄₅N₆O₆B) + H: 561.60. Found: 561.4.

25

Example 8

Preparation of Ac-Phe-Gly-boroArg-OH•benzene sulfonic acid.

Ac-Phe-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid was prepared by coupling Ac-Phe-OH to H-Gly-boroArg-C₁₀H₁₆ using the carbodiimide procedure described in Example 3. FAB/MS calcd for M (C₂₄H₃₉N₆O₅B). 503.51. Found: 503.3.

Ac-Phe-Gly-boroArg-C₁₀H₁₆•benzene sulfonic acid (0.075 g, 0.10 mmoles) was treated with phenyl boronic acid by the procedure in Example 3 to yield Ac-Phe-Gly-boroArg-OH•benzene sulfonic acid. FAB/MS calcd. for the

-19-

pinacol ester, M (C₂₇H₄₃N₄O₅B) + H: 515.48. Found: 515.3.

Example 9

5 Preparation of Ac-(D)Phe-Pro-boroLys-OH•benzene sulfonic acid

The intermediate, NH₂-CH[(CH₂)₄Br]BO₂C₁₀H₁₆•HCl was prepared by the procedure described for the analogous compound, NH₂-CH[(CH₂)₃Br]BO₂C₁₀H₁₆•HCl, in
10 EPA 0 293 88Q. Also by analogous reactions, Ac-(D)Phe-Pro-NH-CH[(CH₂)₄Br]BO₂C₁₀H₁₆, Ac-(D)Phe-Pro-NH-CH[(CH₂)₄N₃]BO₂C₁₀H₁₆, and Ac-(D)Phe-Pro-NH-CH[(CH₂)₄NH₂]BO₂C₁₀H₁₆•benzene sulfonic acid (Ac-(D)Phe-Pro-boroLys-C₁₀H₁₆•benzene sulfonic acid) were prepared.

15 Ac-(D)Phe-Pro-boroLys-C₁₀H₁₆•benzene sulfonic acid (0.50 g, 0.76 mmol) was treated with phenyl boronic acid by the procedure described in Example 3 to yield Ac-(D)Phe-Pro-boroLys-OH•benzene sulfonic acid (0.35 g). FAB/MS calcd. for the pinacol ester, M (C₂₇H₄₃N₄O₅B) +
20 H: 515.48. Found: 515.3.

Example 10

25 Preparation of the Isothiouonium Analog of Ac-(D)Phe-Pro-boroArg-OH

Ac-(D)Phe-Pro-NH-CH[(CH₂)₃-S-C(NH)-NH₂]BO₂-C₁₀H₁₆•HBr. was prepared by the procedure described in EPA 0 293 881. The corresponding bromide was treated with thiourea to yield the desired produce as an
30 amorphous white solid. Anal. Calcd. for C₃₁H₄₇N₅SBBr: C=53.75%, H=6.85%, N=10.11%, B=1.56%. Found: C=53.18%, H=6.68%, N=9.47%, and B=1.50%. FAB/MS calcd. for the pinacol ester, M (C₃₁H₄₆N₅SB) + H: 612.71. Found: 612.36.

35 Ac-(D)Phe-Pro-NH-CH[(CH₂)₃-S-C(NH)-NH₂]BO₂-C₁₀H₁₆•HBr (1.0 g, 1.4 mmol) was allowed to react with phenyl boronic acid by the procedure in Example 3 to

-20-

yield 0.66 g of the desired product, Ac-(D)Phe-Pro-NH-CH[(CH₂)₃-S-C(NH)-NH₂]B(OH)₂·HBr. Anal. Calcd. for C₂₁H₃₃N₅O₅SBBR: C=45.17%, H=5.97%, N=12.55%, and B=1.93%. Found: C=44.78%, H=5.58%, N=12.23%, and B=1.85%. FAB/MS calcd. for the pinacol ester, M (C₂₇H₄₂N₅O₅BS) + H: 560.31. Found: 560.41.

Example 11

Preparation of MeOSuc-Ala-Ala-Pro-(D,L)boroVal-OH

10 The synthesis of MeOSuc-Ala-Ala-Pro-(D,L)boroVal-C₆H₁₂ has been described previously, Kettner and Shenvi J. Biol. Chem. 259: 15106 (1984). The pinacol ester (100 mg, 0.17 mmols) was allowed to react with 5 equivalent of phenyl boronic acid using the conditions described in Example 3. The aqueous phase was 15 evaporated to yield 92 mg of MeOSuc-Ala-Ala-Pro-(D,L)boroVal-OH. NMR indicated only a trace (<10%) of the pinacol group remained. FAB/MS calcd. for the pinanediol ester, M (C₃₀H₄₉N₄O₈B) + H: 605.65. Found: 20 605.4.

Example 12

Preparation of H-(D,L)boroVal-OH

H-(D,L)boroVal-C₆H₁₂·trifluoroacetic acid (100 mg, 0.32 mmols), described in Kettner and Shenvi (1984) was 25 allowed to react with phenyl boronic acid by the procedure in Example 3. H-(D,L)boroVal-OH·trifluoroacetic acid was obtained in a yield of 76 mg. NMR was consistent with the desired structure indicating the complete absence of the pinacol group. 30 FAB/MS calcd. for the pinanediol ester, M (C₁₄H₂₆NO₂B) + H: 252.22. Found: 252.2.

Example 13

Preparation of hydrocinnamoyl-Pro-boroLys-OH benzene sulfonic acid.

35 Hydrocinnamoyl-Pro-boroLys-C₁₀H₁₆ benzene sulfonic acid was prepared by the general procedure described in

-21-

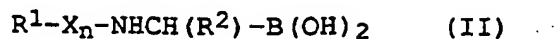
EPA 0 293 881 and was allowed to react with phenyl boronic acid by the procedure in Example 3. The desired product was obtained in a yield of 92%. MS calcd. for $M(C_{19}H_{30}N_3O_4B)+H-2H_2O$: 340.0. Found: 340. Anal

5 Calcd. for $C_{35}H_{50}N_3O_7SB$: c=62.96%, H=7.55%, N=6.29%, B=1.62%. Found: C=62.75%, H=7.47%, N=6.28%, B=1.64%.

-22-

What is Claimed is:

- 5 1. A method for the preparation of a compound of formula (II)



wherein

- 10 R^1 is

- a) hydrogen,
 - b) an N-terminal protecting group,
 - c) $-SO_2(CH_2)_m$ -aryl, wherein aryl is phenyl, naphthyl or biphenyl substituted with one, two or three
- 15 substituents selected from the group consisting of halo (F, Cl, Br, I), $-CN$, C1-C10-alkyl, C3-C8-cycloalkyl, C2-C10-alkenyl, C2-C10-alkynyl, $-OR^7$, $-NO_2$, $-CF_3$, $-S(O)_2R^8$, $-NR^6R^7$, $-COR^7$, $-CO_2R^7$, $-CONR^6R^7$;
 X is a peptide of 1-20 amino acids;

- 20 R^2 is

- a) C1-C10-alkyl,
- b) C2-C10-alkyl-Y,
- c) $-(CH_2)_n$ -aryl, wherein aryl is as defined above;

Y is

- 25
- a) $-NHC(NH)NH_2$,
 - b) $-NH_2$,
 - c) $-SC(NH)NH_2$,
 - d) $-OR^9$,
 - e) $-SR^9$;

- 30 R^5 and R^6 are independently

- a) H,
- b) C1-C8-alkyl,
- c) C1-C8-alkoxy,
- d) C3-C8-cycloalkyl,
- 35 e) $-CO_2R^7$,
- f) $-(CH_2)_m$ -phenyl;

- 40 R^7 is

-23-

- a) H,
- b) phenyl,
- c) benzyl,
- d) C1-C8-alkyl;

5 R⁸ is

- a) phenyl,
- b) C1-C4-alkyl,
- c) C1-C4-alkoxy,
- d) -CF₃;

10 R⁹ is

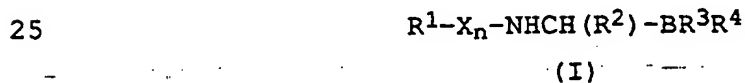
- a) H,
- b) C1-C2-alkyl,
- c) phenyl or phenyl optionally substituted with a substituent selected from the group consisting of halo (F, Cl, Br, I), -CN, C1-C10-alkyl, C3-C8-cycloalkyl, C2-C10-alkenyl, C2-C10-alkynyl, -OR⁷, -NO₂, -CF₃, -S(O)_rR⁸, -NR⁶R⁷, -COR⁷, -CO₂R⁷, -CONR⁶R⁷;

n is 0 or 1;

20 m is 0 to 2;

r is 0 to 2;

comprising suspending a compound of the formula



wherein R¹, R², X, Y, R⁵, R⁶, R⁷, R⁸, R⁹, n, m and r are as defined above; and

30 R³ and R⁴ are

a) C1-C8-alkoxy, or

b) when taken together R³ and R⁴ form a cyclic boronic ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, a heteroatom which can be N, S, or O; R³ and R⁴, independently, are optionally, a heteroatom which can be N, S, or O,

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-24-

in a mixture of water and a water-immiscible organic solvent containing an organic boronic acid acceptor present in an amount equal to at least 1 equivalent of said compound of formula (I),
5 stirring said system for approximately one hour before allowing the reaction mixture to separate into two distinct phases, separating the phases, and recovering the compound of formula (II) from the separated aqueous phase.

10

2. The method of claim 1 wherein the water-immiscible organic solvent is selected from the group consisting of diethyl ether, carbonteterachloride, chloroform, methylene chloride, ethyl acetate,
15 benzene, toluene or hexane.

3. The method of claim 2 wherein the organic boronic acid acceptor is phenyl boronic acid.

20 4. The method of anyone of claims 1 to 3 wherein the amount of organic boronic acid receptor present in the suspending step is in the range of 3 to 5 molar equivalents of the amount of the compound of formula (I) present in said step.

25

5. The method of claim 1 wherein the compound of formula (II) is recovered from the seperated aqueous phase by the evaporation of water from said phase.

30

6. The method of claim 5 wherein the evaporation of water is by means of a rotary evaporator.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/02964

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K1/08 C07K5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 293 881 (E.I. DUPONT NEMOURS AND COMPANY) 7 December 1988 ----	
A	US,A,5 196 948 (D.H.KINDER ET AL.) 21 April 1992 ----	
A	CHEMICAL REVIEWS vol. 89 , 1989 , USA pages 1535 - 1551 D.S.MATTESON 'Alpha- Halo Boronic Esters: Intermediates for Stereodirected Synthesis' -----	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0293881	07-12-88	US-A- 5187157	16-02-93
		AU-B- 623592	21-05-92
		AU-A- 1733288	08-12-88
		CA-A- 1328332	05-04-94
		DE-A- 3878991	15-04-93
		JP-A- 1063583	09-03-89
		US-A- 5242904	07-09-93
US-A-5196948	23-03-93	US-A- 5250720	05-10-93
		JP-A- 3041478	21-02-91
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